Amendments to the Claims

Please amend claims 1, 9, 20, 31, and 50, as indicated in the Listing of Claims.

This Listing of Claims will replace all prior versions and listings of claims in the application:

Listing of Claims:

1. (Currently Amended) A method for inducing differentiation of a stem cell into a neuron, comprising contacting a stem cell with a Hedgehog protein and β-cyclodextrin (βCD) under conditions sufficient to decrease sterol concentration in the cell, wherein the stem cell is an embryonic stem cell or a neuronal stem cell, thereby inducing the stem cell to differentiate into a neuron.

2. (Previously Presented) The method of claim 1, wherein the differentiated cell is a motor neuron.

3. (Previously Presented) The method of claim 1, wherein the differentiated cell is a dopaminergic neuron.

4. (Previously Presented) The method of claim 1, wherein the stem cell is a mammalian cell.

5. (Previously Presented) The method of claim 4, wherein the stem cell is a human cell.

6. (Previously Presented) The method of claim 1, wherein the stem cell is an avian cell.

7. (Previously Presented) The method of claim 1, wherein the hedgehog is protein is an N-terminal fragment of a hedgehog polypeptide.

8. (Previously Presented) The method of claim 1, wherein the βCD is β-methyl CD.

9. (Currently Amended) The method of claim 1, wherein a population of stem cells [[or]] are contacted with Hedgehog protein and βCD, wherein the population of stem cells [[or]] differentiate into a substantially uniform population of differentiated neurons.

10. (Previously Presented) The method of claim 1, wherein the βCD is at a concentration effective for reducing sterol levels to below 40 μg/mg.

11. (Previously Presented) The method of claim 10, wherein the βCD is at a concentration of between about 100 μM and 5 mM.

12. (Previously Presented) The method of claim 11, wherein the βCD is at a concentration of between about 200 μM and 600 μM.

13. (Previously Presented) The method of claim 10, wherein the βCD is used at a concentration of about 400 μM.

14. (Previously Presented) The method of claim 1, wherein the stem cell is a neuronal stem cell.

15. (Previously Presented) The method of claim 1, wherein the Hedgehog protein is a Sonic Hedgehog protein.

16. (Previously Presented) The method of claim 15, wherein the ShhN, is an N-terminal fragment of a Sonic Hedgehog protein.

17. (Previously Presented) A method to alter the responsiveness of a stem cell to a Hedgehog signal, comprising:

a) contacting the stem cell or the progenitor cell with Hedgehog; and

b) contacting the stem cell with βcyclodextrin (βCD) *in vitro* under conditions sufficient to decrease sterol concentration in the cell, thereby altering the responsiveness to a Hh signal.

18. (Previously Presented) The method of claim 17, further comprising detecting expression of a Hedgehog responsive gene.

19. (Previously Presented) The method of claim 17, further comprising detecting expression of a gene whose expression is associated with neuronal differentiation.

20. (Currently Amended) A method for differentiating a population of stem cells into a population of neurons, comprising: contacting a population of stem cells with a differentiation signaling protein under conditions sufficient to induce differentiation, and a sterol-depleting agent under conditions sufficient to decrease sterol concentrations in the population of cells and/or under conditions sufficient to positively effect TGFβ signaling in the population of cells, wherein the stem cells are embryonic stem cells or neuronal stem cells, wherein the differentiation signaling protein is selected from Hedgehog and a Transforming Growth Factor β (TGFβ) family member, and wherein the population of stem cells differentiate into a substantially uniform population of neurons.

21. (Previously Presented) The method of claim 20, wherein the sterol-depleting agent is β-cyclodextrin (βCD).

22. (Previously Presented) The method of claim 21, wherein the stem cell is contacted with βCD under conditions sufficient to positively effect TGFβ signaling.

23. (Previously Presented) The method of claim 22, wherein the stem cell is contacted with a bone morphogenic protein (BMP).

24. (Previously Presented) The method of claim 20, wherein the differentiation signaling protein is a Hedgehog protein.

25. (Previously Presented) The method of claim 24, wherein the differentiation signaling protein is Sonic Hedgehog.

26. (Previously Presented) The method of claim 25, wherein the population of stem cells is contacted with the sterol-depleting agent under conditions sufficient to decrease sterol concentrations.

27. (Previously Presented) The method of claim 26, wherein the sterol-depleting agent is cyclodextrin (CD), nystatin, or filipin.

28. (Previously Presented) The method of claim 24, wherein the differentiation signaling protein is a Hedgehog N-terminal peptide.

29. (Previously Presented) The method of claim 28, wherein the differentiation signaling protein is Sonic Hedgehog N-terminal peptide.

30. (Previously Presented) The method of claim 29, wherein the population of stem cells is contacted with jervine or cyclopamine at a concentration lower than a concentration required to block cholesterol transport.

31. (Currently Amended) A method for differentiating a population of stem cells, comprising:

a) contacting a population of stem cells with a Hedgehog protein under conditions sufficient to induce differentiation wherein the stem cells are embryonic stem cells or neuronal stem cells; and

b) contacting the population of stem cells with β-cyclodextrin under conditions sufficient to decrease sterol concentration in the cells and/or under conditions sufficient to positively effect

TGFβ signaling in the population of cells, wherein the population of stem cells differentiate into a substantially uniform population of differentiated cells.

32. (Previously Presented) The method of claim 31, wherein the population of stem cells or progenitor cells differentiate into a population of cells selected from cells of the central nervous system, intestinal cells, pancreatic cells, lung cells, and retinal cells.

33. (Withdrawn) A method of introducing a cell into a subject, comprising:

a) differentiating a stem cell into a differentiated neuron *in vitro* by the method of claim 1; and

b) introducing the differentiated neuron into the subject.

34. (Withdrawn) The method of claim 33, wherein a substantially uniform population of differentiated neurons produced by the method of claim 1, are introduced into the subject.

35. (Withdrawn) The method of claim 34, wherein the subject is a human.

36. (Withdrawn) The method of claim 35, wherein the human is afflicted with a neurodegenerative disease.

37. (Previously Presented) A substantially uniform population of differentiated neurons produced by the method of claim 1 or 31.

38. (Withdrawn) A method of screening a compound for neuroactivity, comprising:

a) contacting a stem cell with a Hedgehog protein and β-cyclodextrin (βCD) under conditions sufficient to decrease sterol concentration in the cell, thereby inducing the stem cell to differentiate into a neuron;

b) contacting the neuron with a test compound; and

b) detecting an effect of the test compound on the neuron, wherein a test compound that affects the neuron is neuroactive.

39. (Withdrawn) The method of claim 38, wherein the Hedgehog protein is a Sonic Hedgehog protein.

40. (Withdrawn) The method of claim 38, wherein the effect is a change in gene expression in the neuron.

41. (Withdrawn) The method of claim 38, wherein the effect on the neuron is selected from augmenting or stimulating of the action of gamma-aminobutyric acid (GABA), enhancing of the action of serotonin, facilitating the action of dopamine, or activating acetylcholine receptors.

42. (Withdrawn) A method of screening a compound for its effect on neuronal differentiation, comprising:

a) contacting a stem cell with:

i) a Hedgehog protein,

ii) βcyclodextrin (βCD), under conditions sufficient to decrease sterol concentrations in the cell; and

iii) a test compound,

under conditions sufficient to cause the stem cell to differentiate into a neuron in the absence of the test compound; and

b) determining whether the stem cell or the progenitor cell differentiates into a neuron, wherein an absence or presence of differentiation is indicative of a test compound that affects neuronal differentiation.

43. (Withdrawn) The method of claim 42, wherein stem cell is a neural plate explant cell.

44. (Withdrawn) The method of claim 42, wherein the cell is a mammalian cell.

45. (Withdrawn) The method of claim 44, wherein the cell is a human cell.

46. (Withdrawn) The method of claim 42, wherein the Hedgehog protein is a Sonic Hedgehog protein.

47. (Withdrawn) A method for identifying an agent that restores responsiveness to a Hedgehog (Hh) signal in a cell with a loss of responsiveness, comprising:

a) contacting the cell with:

i) B-cyclodextrin (BCD), under conditions sufficient to decrease sterol concentrations in the cell;

ii) an Hh protein; and

iii) a test compound; and

b) determining whether the cell is responsive to the Hh signal as compared with the level of responsiveness in the absence of the compound wherein a higher level of responsiveness to the Hh signal in the presence of the compound identifies a compound that restores responsiveness to the Hh signal.

48. (Withdrawn) The method of claim 47, wherein the isolated cell is a fibroblast.

49. (Withdrawn) The method of claim 47, wherein the Hh protein is a Sonic Hh protein.

50. (Currently Amended) A method for identifying a gene involved in neuronal differentiation, comprising:

a) contacting a stem cell, wherein the stem cell is an embryonic stem cell or a neuronal stem cell, with:

i) a Hedgehog (Hh) protein, under conditions sufficient to cause the stem cell or the progenitor cell to differentiate into a neuron; and

ii) B-cyclodextrin (BCD), under conditions sufficient to decrease sterol concentrations in the cell; and

b) detecting a gene whose expression changes following or during differentiation as compared to before differentiation of the stem cell.

51. (Previously Presented) The method of claim 50, wherein the Hh protein is Sonic Hh protein.

52. (Previously Presented) The method of claim 51, wherein the Sonic Hh N-terminal peptide.

53. (Withdrawn) A method for diagnosing a neurological disorder in a subject, comprising detecting reduced sterol levels or a reduced response to a Hedgehog signal in cells of the subject.

54. (Withdrawn) The method of claim 53, wherein the method detects disorders associated with defects in sterol biosynthesis.

55. (Withdrawn) The method of claim 54, wherein the disorder is detected by detecting a reduced responsiveness to a Hedgehog signal.

56. (Withdrawn) The method of claim 54, wherein the defect is in cholesterol biosynthesis.

57. (Withdrawn) The method of claim 54, wherein the neurological disorder is Smith-Lemli-Optz syndrome (SLOS), desmosterolosis, or lathosterolosis.

58. (Withdrawn) The method of claim 54, wherein the cells are neurons.